

determining algorithm described herein. The following sequences were selected.

- I. N-SQDEVREN-C
- II. N-ELSRESREGR-C
- III. N-RVLEEEENK-C
- IV. N-QKCDSVEE-C
- V. N-EETGINRERKVE-C
- VI. N-EPEIELPREPRNEE-C

B. Materials and methods

- i. Hep G2 cells were seeded into six-well dishes at 30,000 cells/well in MEM-supplemented media with 10% FBS. Cells were grown to near confluency and exposed to
 - I. media alone
 - II. Mevastatin 5 μ Mol (Cholesterol-lowering Statin drug) and,
 - III. HMG CoA Reductase peptides at 1, 10 and 100 μ Mol.
- ii. All treatments were carried out in triplicate. After 24 hrs the media was removed and saved.
- iii. The cells were washed with 1 ml of PBS and combined with the media. The cells were harvested with 1 ml of 0.1N NaOH.
- iv. Samples, both media and cells, were saponified with 0.5 ml 50% KOH, 3 ml ethanol, separately at 80°C for 2 hrs.
- v. After cooling, the samples were extracted twice with petroleum ether and washed with NaOH.
- vi. The samples were dried under nitrogen and suspended in 200 μ l ethanol.
- vii. 50 μ l of this suspension was used for determination of cholesterol. Cholesterol was determined in the samples and standards using colorimetric assay (Sigma Kit).
- viii. Protein was determined by Lowry's method and the results were expressed as μ gm cholesterol/mg protein.

C. Results

i. The following results were obtained:

<u>Peptide</u>	<u>Treatment</u>	<u>Cholesterol</u> <u>Content</u>		<u>Total</u> <u>Cholesterol</u> (μ gm/mg protein)	<u>Cholesterol</u> <u>Lowering in</u> <u>% of Control</u>
		Cell	Media		
	Control	152.1 \pm 39.0	66.5 \pm 6.5	218.6 \pm 35.0	-
	Mevastatin 5 μ M	124.4 \pm 4.4	57.5 \pm 5.2	182.0 \pm 7.0	18.0
1	1 μ M	92.5 \pm 4.7	65.2 \pm 4.0	158.7 \pm 5.6	28.0
	10 μ M	88.6 \pm 1.6	60.6 \pm 0.5	149.1 \pm 2.0	30.0
	100 μ M	94.0 \pm 4.0	61.1 \pm 2.4	155.3 \pm 6.4	30.0
2	1 μ M	94.0 \pm 8.3	64.3 \pm 4.2	158.3 \pm 6.3	29.0
	10 μ M	85.0 \pm 8.4	63.7 \pm 9.5	148.6 \pm 11.8	33.0
	100 μ M	86.0 \pm 4.7	59.0 \pm 6.0	146.9 \pm 8.8	34.0
3	1 μ M	137.3 \pm 4.5	64.0 \pm 2.7	201.3 \pm 3.3	10.0
	10 μ M	135.0 \pm 4.5	65.5 \pm 4.7	200.7 \pm 9.0	10.0
	100 μ M	135.0 \pm 7.0	65.5 \pm 1.4	200.5 \pm 5.4	10.0

Get
Q2

<u>Peptide</u>	<u>Treatment</u>	<u>Cholesterol</u> <u>Content</u>		<u>Total</u> <u>Cholesterol</u> (μ gm/mg protein)	<u>Cholesterol</u> <u>Lowering in</u> <u>% of Control</u>
		Cell	Media		
	Control	151.0 \pm 7.8	50.8 \pm 2.5	202.0 \pm 5.3	-
	Mevastatin 5 μ M	125.0 \pm 4.6	37.5 \pm 3.0	162.6 \pm 7.6	19.0
4	1 μ M	134.7 \pm 3.2	50.4 \pm 2.5	184.3 \pm 5.5	8.0
	10 μ M	126.1 \pm 4.2	49.0 \pm 1.0	175.2 \pm 3.8	10.0
	100 μ M	127.7 \pm 9.3	53.8 \pm 0.5	181.7 \pm 9.3	9.0
5	1 μ M	140.7 \pm 8.6	56.8 \pm 1.0	197.0 \pm 9.2	1.5
	10 μ M	149.0 \pm 4.4	50.7 \pm 1.0	200.0 \pm 5.0	0.0
	100 μ M	161.7 \pm 23.5	48.0 \pm 1.6	210.0 \pm 25.0	0.0
6	1 μ M	144.3 \pm 7.5	47.4 \pm 0.5	192.0 \pm 8.0	4.0
	10 μ M	136.0 \pm 12.2	48.0 \pm 3.4	184.0 \pm 8.4	8.0
	100 μ M	132.0 \pm 4.4	50.4 \pm 1.0	182.4 \pm 4.8	9.0

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REMARKS

Applicant respectfully submits that Example Number 6 does not constitute new matter. Rather, Example Number 6 provides experimental data in support of the presently pending claims.

A Final Office Action relating to the U.S. application serial number 09/232,186 was issued on August 8, 2000. Applicant filed a Notice of Appeal on February 8, 2001, therefore an Appeal Brief was due on April 8, 2001. Applicant hereby requests a THREE-MONTH extension time in lieu of an Appeal Brief; i.e., the filing of this continuation.

The Commissioner is hereby authorized to charge payment of the 37 C.F.R. § 1.136(a) extension fee to